

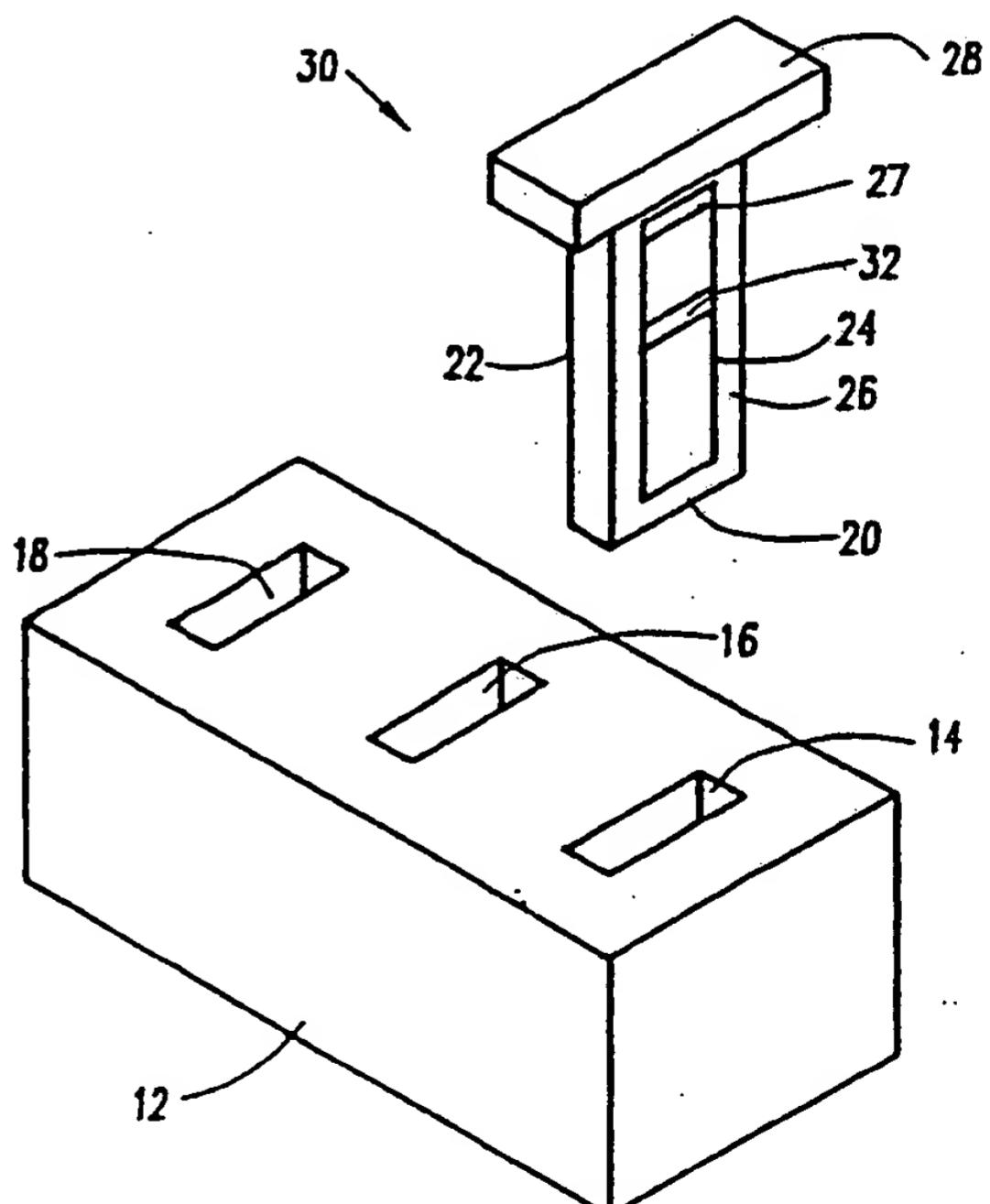
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(54) Title: METHOD AND APPARATUS FOR DETECTION OF NUCLEIC ACID SEQUENCES

(57) Abstract

Apparatus for transport of molecules including nucleic acid sequences in a bibulous carrier comprising a dry bibulous carrier defining a capillary transport path which supports the transport of the molecules when contacted with a solution containing the molecules.



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1 Method and apparatus for detection of nucleic acid sequences
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4 FIELD OF THE INVENTION

5 The invention relates to apparatus and methods for
6 separation of target molecules including target
7 nucleic acid sequences from oligonucleotides, and
8 nucleotides and concentration and detection of the
9 molecules.

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14 BACKGROUND OF THE INVENTION

15 The use of amplification techniques in a procedure
16 for detection of a target molecules that include target
17 nucleic acid sequences is well known in the art.
18 Typically, this procedure includes enzymatic
19 amplification of target nucleic acid sequences and
20 detection of the target molecules by gel
21 electrophoresis followed by Southern blot procedures.

22 A number of solid phase capture assays have also
23 been developed to simplify the procedures for detection
24 of target molecules including nucleic acid sequences.

25 In these procedures two ligands are typically
26 incorporated within amplified target nucleic acid
27 sequences. A first ligand is used to capture, on a
28 solid matrix, the target molecules that include the
29 amplified target nucleic acid sequences and a second
30 ligand is used to detect the target molecules by the
31 binding of a signal producing reagent to this second
32 ligand.

33 Solid phase affinity capture assays, however,
34 require an extended reaction time to capture a high
35 proportion of target molecules in a reaction mixture
36 (Sauvaigo et al., Nucleic Acid Research, 1990, Vol. 18,
37 pp. 3175 - 3182). Furthermore, when capture is mediated
38 by amplification primers incorporating a solid phase

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1 affinity ligand, the sensitivity of the assay may be
2 adversely effected by competition between free primers
3 and primers incorporated in the target nucleic acid
4 sequences.

5 The use of chromatography as a separation and
6 concentration procedure is well known in the art. It
7 has been reported that whereas DNA molecules are
8 chromatographically mobile on moistened paper they fail
9 to migrate when solutions are applied to dry paper
10 (Bendich et al., Arch. Biochem. Biophys., 1961, 94,
11 417-423).

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SUMMARY OF THE INVENTION

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4 One object of the present invention is to provide
5 a method and apparatus for capillary transport of
6 molecules including nucleic acid sequences.

7 Another object of the present invention is to
8 provide a method and apparatus for concentration of
9 target molecules including target nucleic acid
10 sequences in a liquid sample.

11 A further object of the present invention is to
12 provide a method and apparatus for the separation of
13 target molecules including target nucleic acid
14 sequences from nucleotides and oligonucleotides.

15 Another object to the present invention is to
16 provide a method for the detection of target molecules
17 including specific nucleic acid sequences.

18 There is thus provided in accordance with the
19 present invention apparatus for transport of molecules
20 including nucleic acid sequences in a bibulous carrier
21 comprising a dry bibulous carrier defining a capillary
22 transport path which supports the transport of the
23 molecules when contacted with a solution containing the
24 molecules.

25 In accordance with a preferred embodiment of the
26 invention apparatus for concentration of target
27 molecules in a liquid sample is provided including the
28 dry bibulous carrier wherein the target molecules
29 include target nucleic acid sequences and are
30 transported within the bibulous carrier by capillary
31 action when a portion of the dry bibulous carrier
32 contacts the liquid sample containing the target
33 molecules, and at least one capture reagent immobilized
34 in at least one capture zone on the dry bibulous
35 carrier downstream of a contact portion of the bibulous
36 carrier wherein the at least one capture reagent is
37 capable of capturing the target molecules.

38 There is also provided in accordance with the

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1 present invention apparatus for separation of target
2 molecules, including target nucleic acid sequences,
3 from non-target nucleotides and oligonucleotides in a
4 liquid sample containing the target molecules and the
5 non-target nucleotides and oligonucleotides comprising,
6 a vessel containing a compound that binds the non-
7 target oligonucleotides, and apparatus for transporting
8 the target molecules from the vessel by capillary
9 action.

10 In accordance with a preferred embodiment of the
11 invention the dry bibulous carrier is a nitrocellulose
12 membrane wherein the absorption sites have been blocked
13 to facilitate capillary transport of the target
14 molecules.

15 In accordance with another preferred embodiment
16 of the invention the dry bibulous carrier is supported
17 by a rigid frame.

18 In accordance with still another preferred
19 embodiment of the invention an absorbent pad is fixed
20 to the dry bibulous carrier downstream from the at
21 least one capture zone to facilitate capillary
22 transport of a liquid through the dry bibulous carrier.

23 In accordance with yet another a preferred
24 embodiment of the invention the absorption sites of the
25 nitrocellulose membrane are blocked by compounds
26 selected from a group comprising macromolecules,
27 detergents and combinations thereof.

28 In accordance with still another preferred
29 embodiment of the invention the macromolecules include
30 proteins.

31 In accordance with still a further preferred
32 embodiment of the invention the at least one capture
33 reagent includes an antibody to a modified portion of
34 the target nucleic acid sequences.

35 In accordance with another preferred embodiment of
36 the invention the at least one capture reagent
37 includes at least one nucleic acid capture reagent
38 including nucleic acid probe sequences complementary to

- 1 at least part of the target nucleic acid sequences.
- 2 In accordance with still another preferred embodiment of the invention the nucleic acid probe sequences include DNA sequences.
- 5 In accordance with yet another preferred embodiment of the invention the nucleic acid probe sequences include RNA sequences.
- 8 In accordance with a further preferred embodiment of the invention the target molecules include target nucleic acid sequences comprising more than 30 base pairs.
- 12 In accordance with another preferred embodiment of the invention wherein the target molecules including nucleic acid sequences include a nucleic acid product of an enzymatic amplification reaction and incorporate at least one pair of oligonucleotide primers.
- 17 In accordance with still another preferred embodiment of the invention the at least one pair of primers include primers for a polymerase chain reaction (PCR).
- 21 In accordance with a further preferred embodiment of the invention the at least one pair of primers include primers for a ligase chain reaction (LCR).
- 24 In accordance with yet a further preferred embodiment of the invention at least a second primer of the at least one pair of primers includes an oligonucleotide bearing a ligand which binds to at least one capture reagent whereby the target molecules which include the at least one primer bearing the ligand may be bound to the at least one capture reagent.
- 32 In accordance with still a further preferred embodiment of the invention the ligand which binds to at the least one capture reagent includes an antigenic epitope.
- 36 In accordance with another preferred embodiment of the invention the ligand which binds to the at least one capture reagent includes at least one sulfonated

1 cytosine.

2 In accordance with yet another preferred
3 embodiment of the invention the compound includes gel
4 filtration particles too large to be transported by the
5 apparatus for transporting.

6 In accordance with a yet another preferred
7 embodiment of the invention the non-target
8 oligonucleotides include oligonucleotide primers not
9 incorporated in the target nucleic acid sequences.

10 In accordance with a further preferred embodiment
11 of the invention the compound includes a matrix unable
12 to be transported by the means for transporting and
13 wherein the compound hybridizes to the non-target
14 oligonucleotide.

15 There is also provided in accordance with the
16 present invention a method for transport of molecules
17 including nucleic acid sequences in a bibulous carrier
18 including the steps of, providing a dry bibulous
19 carrier defining a capillary transport path which
20 supports the transport of molecules including nucleic
21 acid sequences, and contacting the dry bibulous carrier
22 with a solution containing molecules including nucleic
23 acid sequences.

24 There is additionally provided in accordance with
25 the present invention a method for concentration of
26 molecules, including nucleic acid sequences, in a
27 liquid sample including the steps of, providing a dry
28 bibulous carrier wherein the molecules are target
29 molecules including target nucleic acid sequences and
30 wherein the molecules are transported within the
31 bibulous carrier by capillary action when a portion of
32 the dry bibulous carrier contacts the liquid sample
33 containing the molecules, contacting a portion of the
34 dry bibulous carrier with the liquid sample containing
35 the target molecules wherein the dry bibulous carrier,
36 when wet, defines a liquid transport path which
37 supports the transport of molecules including nucleic
38 acid sequences, transporting the target molecules along

1 the liquid transport path, and capturing the target
2 molecules with at least one capture reagent immobilized
3 in at least one capture zone on the dry bibulous
4 carrier downstream of the portion of bibulous carrier
5 contacting the liquid sample.

6 There is further provided according to the present
7 invention a method for separation of target molecules,
8 including target nucleic acid sequences, from non-
9 target nucleotides and oligonucleotides, in a liquid
10 sample containing the target molecules and the non-
11 target nucleotides and oligonucleotides including the
12 steps of, providing a vessel containing a compound that
13 binds the non-target nucleotide and oligonucleotide
14 sequences, adding the liquid sample which includes the
15 target molecules and the non-target nucleotide and
16 oligonucleotides, and transporting the target molecules
17 by capillary action.

18 There is also provided in accordance with the
19 present invention apparatus for separation of target
20 molecules, including target nucleic acid sequences,
21 from non-target nucleotides and oligonucleotides in a
22 liquid sample containing the target molecules and the
23 non-target nucleotides and oligonucleotides,
24 concentration of the target molecules, and detection of
25 the concentrated target molecules including, a vessel
26 apparatus defining a plurality of wells including a
27 first portion of the plurality of wells containing a
28 compound that binds the non-target oligonucleotides and
29 wherein the liquid sample may be added to the first
30 portion of the plurality of wells, a dry bibulous
31 carrier defining a liquid transport path from the
32 vessel, that, when wet, supports the transport of the
33 target molecules, wherein the target molecules are
34 transported within the bibulous carrier by capillary
35 action when a contact portion of the dry bibulous
36 carrier contacts the liquid sample containing the
37 target molecules, at least one capture reagent capable
38 of capturing the target molecules wherein the at least

1 one capture reagent is immobilized in at least one
2 capture zone on the dry bibulous carrier downstream of
3 the contact portion of the bibulous carrier, and
4 apparatus for detecting the captured target molecules.

5 There is further provided in accordance with the
6 present invention a method for concentration and
7 detection of target nucleic acid sequences, in a
8 liquid sample including the steps of, providing a dry
9 bibulous carrier wherein the target nucleic acid
10 sequences are transported within the bibulous carrier
11 by capillary action when a portion of the dry bibulous
12 carrier contacts the liquid sample containing the
13 target nucleic acid sequences, contacting a portion of
14 the dry bibulous carrier with the liquid sample
15 containing the target nucleic acid sequences wherein
16 the dry bibulous carrier, when wet, defines a liquid
17 transport path which supports the transport of the
18 target nucleic acid sequences, transporting the target
19 nucleic acid sequences along the liquid transport path
20 and capturing the target nucleic acid sequences by
21 hybridization with at least one nucleic acid capture
22 reagent immobilized in at least one capture zone on the
23 dry, bibulous carrier downstream of the portion of
24 bibulous carrier contacting the liquid sample. There
25 is still further provided in accordance with the
26 present invention apparatus for concentration and
27 detection of target nucleic acid sequences including,
28 a vessel apparatus defining a plurality of wells, a
29 dry bibulous carrier defining a liquid transport path
30 from the vessel that when wet supports the transport of
31 the target nucleic acid sequences wherein the target
32 nucleic acid sequences are transported within the
33 bibulous carrier by capillary action when a contact
34 portion of the dry bibulous carrier contacts the liquid
35 sample containing the target nucleic acid sequences, at
36 least one nucleic acid capture reagent including
37 nucleic acid probe sequences for capturing the target
38 nucleic acid sequences by hybridization and wherein

1 the at least one nucleic acid capture reagent is
2 immobilized in a capture zone on the dry bibulous
3 carrier downstream of the contact portion of the
4 bibulous carrier, and apparatus for detecting the
5 captured the target nucleic acid sequences.

6 In accordance with a preferred embodiment of the
7 invention the apparatus for detecting includes a
8 bibulous carrier upon which target molecules bearing a
9 ligand which binds to a signal producing reagent are
10 immobilized, and apparatus for contacting the target
11 molecules bearing the ligand with the signal producing
12 reagent to produce a sensible signal indicating the
13 detection of the target molecules.

14 In accordance with a further preferred embodiment
15 of the invention the apparatus for detecting includes a
16 bibulous carrier upon which target molecules bearing a
17 ligand which binds to a signal producing reagent are
18 immobilized, and apparatus for contacting the target
19 molecules bearing the ligand with the signal producing
20 reagent which react with a color developing reagent to
21 produce a sensible signal indicating the detection of
22 the target molecules.

23 In accordance with another preferred embodiment of
24 the invention the target nucleic acid sequences are
25 the product of an enzymatic amplification reaction and
26 incorporate at least one pair of oligonucleotide
27 primers.

28 In accordance with yet another preferred
29 embodiment of the invention the non-target
30 oligonucleotides include oligonucleotide primers not
31 incorporated in the target nucleic acid sequences.

32 In accordance with still another preferred
33 embodiment of the invention the at least two sets of
34 primers include primers for a polymerase chain reaction
35 (PCR).

36 In accordance with a further preferred embodiment
37 of the invention the at least one pair of primers
38 include primers for a ligase chain reaction (LCR).

1 In accordance with still a further preferred
2 embodiment of the invention a second primer of the at
3 least one pair of oligonucleotide primers includes a
4 ligand which binds to the at least one capture reagent
5 whereby the target molecules that include the ligand
6 may be bound to the at least one capture reagent.

7 In accordance with yet a further preferred
8 embodiment of the invention the ligand which binds to
9 the at least one capture reagent includes an antigenic
10 epitope.

11 In accordance with another preferred embodiment
12 of the invention the ligand which binds to the at least
13 one capture reagent includes at least one sulfonated
14 cytosine.

15 In accordance with still another preferred
16 embodiment of the invention a first primer of the at
17 least one pair of primers includes a ligand which binds
18 to a signal producing reagent whereby the target
19 molecules that include the ligand may be detected by
20 the presence of a signal produced by the signal
21 producing reagent.

22 In accordance with a further preferred embodiment
23 of the invention the first primer of the at least one
24 pair of primers includes a ligand which binds to a
25 signal producing reagent whereby the target molecules
26 that include the ligand may be detected by the
27 presence of a signal produced by the signal producing
28 reagent after contacting a signal developing reagent.

29 In accordance with yet another preferred
30 embodiment of the invention the ligand which binds to
31 the signal producing reagent includes biotinylated
32 nucleotide sequences. In accordance with a further
33 preferred embodiment of the invention the signal
34 producing reagent includes streptavidin linked to
35 colored latex
36 beads.

37 In accordance with another preferred embodiment of
38 the invention the signal produced by the signal

1 producing reagent after contacting the signal
2 developing reagent includes a streptavidin-alkaline
3 phosphatase conjugate.

4 In accordance with another preferred embodiment of
5 the invention the first portion of wells also contains
6 the signal producing reagent.

7 In accordance with yet a further preferred
8 embodiment of the invention the plurality of wells
9 additionally includes a second portion of the wells
10 containing a washing solution.

11 In accordance with still another preferred
12 embodiment of the invention the plurality of wells also
13 includes a third portion of the wells containing a
14 signal developing reagent solution.

15 In accordance with yet another preferred
16 embodiment of the invention the dry bibulous carrier
17 includes at least one strip.

18 In accordance with a further preferred embodiment
19 of the invention the plurality of wells include a
20 first portion of wells containing a sample to be tested
21 for the target nucleic acid sequences.

22 In accordance with another preferred embodiment of
23 the invention the plurality of wells additionally
24 include a second portion of the wells containing the
25 signal producing reagent.

26 In accordance with yet another preferred
27 embodiment of the invention the plurality of wells
28 additionally includes a third portion of wells
29 containing a washing solution.

30 In accordance with still another preferred
31 embodiment of the invention the plurality of wells
32 additionally includes a fourth portion of wells
33 containing a signal developing reagent.

34 In accordance with a further preferred embodiment
35 of the invention each of the first portion of wells are
36 adapted to receive the contact portion of each strip to
37 permit transport of the target molecules to the at
38 least one capture zone where they are captured.

1 In accordance with still a further preferred
2 embodiment of the invention each of the second portion
3 of wells are adapted to receive the contact portion of
4 each strip for washing the strip to remove non-
5 specifically captured compounds after immobilization of
6 the target molecules in the at least one capture zone.

7 In accordance with yet a further preferred
8 embodiment of the invention each of the third portion
9 of wells is adapted to receive an entire strip.

10 In accordance with another preferred embodiment of
11 the invention the apparatus for contacting includes, at
12 least one of the third portion of wells containing a
13 signal producing reagent solution, and at least one
14 strip after immobilization of the target nucleic acid
15 in the at least one capture zone wherein the entire
16 strip is in contact with a signal developing reagent
17 solution permitting contact of the signal developing
18 reagent with the at least one capture zone.

19 In accordance with yet another preferred
20 embodiment of the invention each of the first portion
21 of wells is adapted to receive the contact portion of
22 each strip to permit transport of the target nucleic
23 acid sequences to the at least one capture zone where
24 they are captured.

25 In accordance with still another preferred
26 embodiment of the invention each of the second portion
27 of wells is adapted to receive the contact portion of
28 each strip to permit transport of the signal producing
29 reagent to the at least one capture zone where the
30 signal producing reagent is bound to the ligand borne
31 on the target nucleic acid sequences.

32 In accordance with a further preferred embodiment
33 of the invention each of the third portion of wells is
34 adapted to receive the contact portion of each strip
35 for washing the strip to remove non-specifically
36 captured compounds after immobilization of the target
37 nucleic acid sequences in the at least one capture
38 zone.

1 In accordance with yet a further preferred
2 embodiment of the invention the apparatus for
3 contacting includes, at least one of the fourth portion
4 of wells containing a signal developing reagent, and at
5 least one strip after immobilization of the target
6 nucleic acid sequences in the at least one capture
7 zone wherein the entire strip is in contact with the
8 signal developing reagent solution permitting contact
9 of the signal developing reagent with the at least one
10 capture zone.

11 In accordance with a still further preferred
12 embodiment of the invention each of the fourth portion
13 of wells is adapted to receive an entire strip.

14 There is also provided in accordance with the
15 present invention a method for the detection of a
16 specific nucleic acid sequence including the steps of,
17 amplifying by an enzymatic reaction at least a portion
18 of an original nucleic acid sequence to produce target
19 molecules including nucleic acid sequences which are
20 specific to the at least a portion of the original
21 nucleic acid sequence, separating the target molecules
22 from non-target nucleotides and oligonucleotides
23 including the steps of, providing a vessel containing a
24 substrate that binds the non-target nucleotides and
25 oligonucleotides, adding a liquid sample which includes
26 the target molecules and the non-target
27 nucleotides and oligonucleotides, and transporting the
28 target molecules by capillary action, concentrating the
29 target molecules including the steps of, providing a
30 dry bibulous carrier wherein the target molecules are
31 transported within the bibulous carrier by capillary
32 action when a portion of the dry bibulous carrier
33 contacts the liquid sample containing the target
34 molecules, contacting a portion of the dry bibulous
35 carrier with the liquid sample containing the target
36 nucleic acid sequences wherein the dry bibulous
37 carrier, when wet, defines a liquid transport path
38 which supports the transport of the target molecules

1 transporting the target molecules along the liquid
2 transport path and capturing the target molecules
3 with at least one capture reagent immobilized in at
4 least one capture zone on the dry bibulous carrier
5 downstream of the portion of bibulous carrier
6 contacting the liquid sample, and detecting the target
7 molecules by contacting target molecules having a
8 ligand which binds to a signal producing reagent and
9 are immobilized on a bibulous carrier with a signal
10 producing reagent to produce a sensible signal.

11 There is also provided in accordance with the
12 present invention a method for the detection of a
13 specific nucleic acid sequence comprising the steps of,
14 amplifying by an enzymatic reaction at least a portion
15 of an original nucleic acid sequence to produce target
16 nucleic acid sequences which are specific to the at
17 least a portion of the original nucleic acid sequence,
18 providing a liquid sample which includes the target
19 nucleic acid sequences, transporting the target nucleic
20 acid sequences by capillary action, concentrating the
21 target nucleic acid sequences including the steps of
22 providing a dry bibulous carrier wherein the target
23 nucleic acid sequences are transported within the
24 bibulous carrier by capillary action when a portion of
25 the dry bibulous carrier contacts the liquid sample
26 containing the target nucleic acid sequences,
27 contacting a portion of the dry bibulous carrier with
28 the liquid sample containing the target nucleic acid
29 sequences wherein the dry bibulous carrier, when wet,
30 defines a liquid transport path which supports the
31 transport of the target nucleic acid sequences, and
32 transporting the target nucleic acid sequences along
33 the liquid transport path, capturing the target
34 nucleic acid sequences with at least one nucleic acid
35 capture reagent immobilized in at least one capture
36 zone on the dry bibulous carrier downstream of the
37 portion of bibulous carrier contacting the liquid
38 sample and detecting the target nucleic acid sequences

1 by contacting target nucleic acid sequences having a
2 ligand which binds to a signal producing reagent and
3 are immobilized on a bibulous carrier with a signal
4 developing reagent to produce a sensible signal.

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1 BRIEF DESCRIPTION OF THE DRAWINGS

2 The present invention will be understood and
3 appreciated more fully from the following detailed
4 description taken in conjunction with the drawings in
5 which:

6 Fig. 1 is a front view pictorial illustration of
7 apparatus for separation of a target nucleic acid
8 sequences from non-target nucleotides and
9 oligonucleotides in a liquid sample, concentration of
10 the target nucleic acid sequences, and detection of the
11 concentrated target nucleic acid sequences constructed
12 and operative in accordance with the present invention
13 and shown before use;

14 Fig. 2 is a front view pictorial illustration of
15 the apparatus of Fig. 1 shown during use;

16 Fig 3 is a front pictorial view of an alternative
17 embodiment of the apparatus of Fig. 1 shown before use;
18 and

19 Fig. 4 is a front pictorial view illustration of
20 the apparatus of Fig. 3 shown during use.

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2 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

3

4 Reference is now made to Figs. 1 - 4 which
5 illustrates apparatus 10 for separation of a target
6 molecules including target nucleic acid sequences from
7 non-target nucleotides and oligonucleotides in a liquid
8 sample, concentration of the target molecules, and
9 detection of the concentrated target molecules
10 constructed and operative in accordance with a
11 preferred embodiment of the present invention.

12 Apparatus 10 includes vessel apparatus 12
13 fabricated from a non-porous material such as
14 polystyrene and including one or more of a plurality of
15 wells such as wells 14, 16 and 18. The wells, such as
16 wells 14, 16, and 18, are approximately 1 cm in length,
17 0.5 cm in width, and 2.5 cm in depth, and are sized to
18 receive the a contact portion 20 of a strip 22.

19 The strip 22 includes a bibulous carrier 24
20 typically embodied in a mylered nitrocellulose membrane
21 approximately 3.0 cm in length and 0.5 cm in width and
22 having a pore size of 3 - 5 microns which may be
23 surrounded by a support frame 26. The support frame 26
24 is fabricated from a non-porous material such as
25 polystyrene, and bibulous carrier 24 may be mounted in
26 frame 22 by any convenient means such as gluing. An
27 absorbent pad 27 approximately 2 cm in length and 0.5
28 cm in width, fabricated from an absorbent material such
29 as Whatman 3MM paper (commercially available from
30 Whatman, Maidstone, U.K.) is attached to the end of the
31 strip 22 opposite the contact portion 20 by any
32 convenient means such as gluing. The end of strip 22
33 is also attached to a handle 28 by any convenient means
34 such as gluing. The handle 28 is fabricated from a non-
35 porous material such as polystyrene. At least one strip
36 22 is attached to the handle 28 to form a test member
37 30.

38 A single capture reagent is typically immobilized

1 on the bibulous carrier 24 in the central area of the
2 bibulous carrier, to form a capture zone 32. Although a
3 single capture reagent is typically employed, multiple
4 capture reagents may be used to form multiple capture
5 zones on a single bibulous carrier.

6 The single capture reagent, typically an anti-
7 sulfonated DNA antibody or a nucleic acid
8 complementary to at least part of the target nucleic
9 acid sequence, is typically immobilized by absorption
10 on the nitrocellulose membrane.

11 Wells 14 typically contain an enzymatic
12 amplification reaction mixture. In addition, when the
13 capture reagent is an anti-sulfonated DNA antibody the
14 wells 14 also typically contain gel filtration
15 particles (not shown), typically Sephadex G-100
16 (Pharmacia, Uppsala, Sweden) gel filtration particles.
17 The gel filtration particles are sized to be too large
18 to be transported by capillary action in the bibulous
19 carrier 24.

20 The procedure used to detect specific nucleic acid
21 sequences using apparatus 10 typically includes the
22 enzymatic amplification of the specific nucleic acid
23 sequence using Polymerase Chain Reaction (PCR) or
24 Ligase Chain Reaction (LCR) employing at least one pair
25 of primers. At least a first primer of the at least one
26 pair of primers of these reactions bears an affinity
27 ligand, typically biotin, which binds to a signal
28 producing reagent, typically a streptavidin alkaline
29 phosphatase conjugate. In addition, when the capture
30 reagent is an anti-sulfonated DNA antibody at least one
31 second primer of the at least one pair of primers for
32 the enzymatic amplification bears an affinity ligand,
33 typically a sulfonated cytosine, which is bound by the
34 capture reagent of the capture zone 32. After a number
35 of amplification cycles, typically between 1 and 50
36 cycles, an aliquot of a reaction mixture is assayed
37 using apparatus 10.

38 When the capture reagent is an anti-sulfonated DNA

1 antibody, an aliquot of the reaction mixture containing
2 target nucleic acid sequences, oligonucleotide primers,
3 and nucleotides, typically between 1 and 20 μ l is added
4 to well 14. Approximately 30 μ l of a solution
5 containing a signal producing reagent, typically
6 streptavidin alkaline phosphatase conjugate in a TPG
7 running buffer (0.3% Tween 20 and 1% gelatin in PBS),
8 is also added to well 14 and the contact portion 20 of
9 strip 22 is placed in well 14 in contact with the
10 reaction mixture. The reaction mixture containing the
11 target molecules including the nucleic acid sequences
12 is carried through the bibulous carrier 24 by
13 capillary transport, past the capture zone 32 where
14 the target molecules are captured by the capture
15 reagent, to the absorbent pad 27.

16 After about 10 minutes most of the molecules that
17 include labeled nucleic acid sequences (typically more
18 than 80% of the labeled molecules) are captured in the
19 capture zone 32. The contact portion 20 of the strip 22
20 is then removed from the well 14 and placed in the well
21 16.

22 The well 16 typically contains about 50 μ l of TP
23 buffer (0.3% tween in PBS) which is carried through
24 the bibulous carrier 24 to the capture zone to remove
25 non-specifically captured compounds which may interfere
26 with the detection of the target nucleic acid sequence.
27 After about 10 minutes strip 22 is removed from well 16
28 and immersed in well 18.

29 Well 18 contains about 300 μ l of signal developing
30 reagent solution, typically a ChemiprobeTM solution
31 containing the chromogenic substrate, BCIP/NBT,
32 commercially available from Organics Ltd., Yavne
33 Israel). This solution covers the capture zone 32. The
34 signal producing reagent, alkaline phosphatase, which
35 is attached to the labeled molecules in the capture
36 zone 32 then converts the chromogenic substrate to a
37 precipitable color which is a sensible signal
38 indicating detection of the target nucleic acid

1 sequences.

2 When the capture reagent is a nucleic acid
3 complementary to at least part of the target nucleic
4 acid sequence an aliquot of the reaction mixture is
5 typically diluted with a hybridization solution
6 typically composed of 0.6M NaCl, 20mM phosphate buffer,
7 pH 7.5, 0.02% Ficoll 400 (Sigma, St. Louis, MO, USA),
8 0.02% gelatin and 1% PVP. The sample is then typically
9 boiled and chilled immediately and an aliquot of each
10 solution transferred to the wells 14 of the apparatus
11 12. The contact portion 20 of each strip 22 is then
12 typically brought into contact with the solution in the
13 wells 14.

14 Apparatus 10 is then typically placed in a humid
15 incubator for approximately 25 minutes and the solution
16 allowed to migrate through the nitrocellulose strips
17 forming the bibulous carrier 24. The solution
18 containing the target molecules including the nucleic
19 acid sequences is carried through the bibulous carrier
20 24 by capillary transport to the absorbent pad 27 and
21 past the capture zone 32 where the target molecules are
22 captured by the nucleic acid complementary to the
23 target nucleic acid sequence.

24 The strips 22 are then typically transferred to
25 wells 16 containing streptavidin alkaline phosphatase
26 conjugate. The strips 22 are then typically transferred
27 to wells 18 containing a solution including 150 μ l of
28 0.3% Tween 20 in PBS and the contact portion 20 of the
29 strip 22 was brought into contact with the solution for
30 approximately 15 minutes.

31 Finally the strips 22 are then typically
32 completely immersed in a ChemiProbeTM BCIP/NBT solution
33 in a set of wells not shown in the figures for
34 approximately 20 minutes to provide a substrate for a
35 chromogenic reaction. A blue colored signal in the
36 capture zone 32 of strip 22 indicating the presence the
37 target molecules.

38 As can be seen in Figs. 3 and 4 more then one

1 strip 22 can be attached to handle 28 to permit more
2 that one assay to carried out at the same time.
3 Reference is now be made to the following examples
4 which, together with Figs. 1 - 4 illustrate the
5 invention.

6

7

8

9

EXAMPLE 110 TRANSPORT AND CONCENTRATION OF DNA ON NITROCELLULOSE
11

12 a) Sequence synthesis and labeling of primers
13 Primers were selected in the gene of HIV-1 and had the
14 following sequences:

15 Primer 3

16 5'TGGGAAGTTCAATTAGGAATACCAC

17 Primer 3'5'TGGGAAGTTCAATTAGGAATA

18

19 Primer 4

20 5'CCTACATACAAATCATCCATGTATTG

21

22 The primers were synthesized on Applied Biosystems
23 380A DNA Synthesizer (Applied Biosystems, Hayward, CA,
24 USA) and purified using OPC rapid purification
25 cartridges (Applied Biosystems, CA, USA).

26

27 Primer sulfonation

28 The primer 3' was synthesized with a 13 mere
29 Polycytosine Tail at the 5' end. These primer was then
30 sulfonated according to the protocol described in the
31 ChemiProbeTM kit (commercially available from Organics
32 Ltd.).

33 100 μ l of C Tail primer (0.5 mg/ml) was mixed with
34 50 μ l of solution A of the ChemiProbeTM Kit (4M sodium
35 bisulfite) and 12.5 μ l of solution B of the
36 ChemiProbeTM Kit (1M methoxyamine) and incubated
37 overnight at 20°C. Sulfonated oligonucleotides were
38 then desalting by centrifuging through a 2 ml bed of

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1 Sephadex G-50 spin column.

2

3 Primer biotinylation

4 Primer 4 was synthesized in the 5' end with a 12 mere
5 polycytosine in which 4 cytosine nucleotides were
6 replaced by N⁴-LCA-5-methyldeoxycytidine (American
7 Bionetics, Hayward, CA, USA) as follows CCCCCCCCCCCC,
8 where C indicates the modified cytosine. These
9 oligonucleotides were purified by acrylamide gel
10 according to the procedures described by Maniatis, T.
11 et al., Molecular cloning: a laboratory manual, 1989, p
12 646, Cold Spring Harbor Laboratory, Cold Spring Harbor,
13 N.Y. the teachings of which are herein incorporated by
14 reference.

15 The purified oligonucleotides were then
16 biotinylated according to the following procedure:

17 10 nmole of desiccated primers were dissolved in
18 50 μ l of 100 mM Borate Buffer and added to 50 μ l of
19 dimethyl formamide (DMF) containing 0.1 mg of biotin N
20 Hydroxy succinimide (Pierce, Rockford, Ill. USA). This
21 solution was then incubated overnight at 20°C and then
22 purified through a Nensorb 20 column (Du Pont Company,
23 Wilmington, DE, USA) according to the instructions of
24 the supplier. The primers were then concentrated by
25 evaporation and resuspended with water to the original
26 concentration.

27

28 b) Amplification of the HIV sequence 100 μ l of a
29 mixture containing 1 μ g of extracted DNA from a
30 positive HIV sample (extraction procedure according to
31 Edwards et al., The Journal of Pediatrics, 1989, vol.
32 45, pp 200-203) the teachings of which are herein
33 incorporated by reference. 100 pmole of each primer P3
34 and P4, 0.25 mM of the four deoxynucleotide
35 triphosphate (dNTP), 10 μ l 10X Taq Buffer (Promega ,
36 Madison, Wisconsin. USA) and 2.5 U of Tag polymerase
37 (Promega) was amplified under the following conditions
38 on a programmable Grant (Cambridge, U.K.) water bath.

1 A first DNA denaturing step of 5 minutes at 94°C
2 was followed by 30 cycles of 1 minute denaturing at
3 94°C. 1 minute DNA annealing at 52°C and 1 minute DNA
4 elongation at 72°C. The amplification was ended with a
5 seven minute elongation step at 72°C.

6 A second amplification was performed for 20 cycles
7 under the same conditions as the first amplification
8 but using the labeled biotinylated and sulfonated
9 primers described above. The DNA template employed was
10 1 μ l of the first PCR mixture diluted in 100 μ l of a
11 mixture containing 100 pmoles of each labeled primer,
12 0.25 mM of the four deoxynucleotide triphosphate, 10 μ l
13 of 10X Taq buffer (Promega) and 2.5 U of Taq polymerase
14 (Promega). Primers were excluded from the PCR Product
15 by mixing 100 μ l of the reaction mixture with 60 μ l of
16 polyethylene glycol (PEG) 4000 (Sigma, St. Louis. MO,
17 USA) in 2.5 M NaCl solution. This mixture was then
18 incubated for one hour at 4°C. Then, after 10 minutes
19 of centrifugation at 10,000 xg at 4°C the supernatant
20 was discarded and the pellet was resuspended in 100 μ l
21 of water.

22

23 c) Preparation of nitrocellulose backed strips

24 1. Mylered Nitrocellulose (pore size 3 μ)
25 (Schleicher & Schuell, Dussel, Germany) were cut into
26 lengths of 0.5 x 3.0 cm to form the bibulous carrier 24
27 of the apparatus of Figs. 1 - 4. The bibulous carriers
28 24 formed strips 22. One microliter of purified mouse
29 monoclonal anti-modified DNA (2 mg/ml), commercially
30 available from Organics Ltd., catalog no. 10793010,
31 supplemented with 1% sucrose in phosphate buffered
32 saline (PBS) was embedded in the middle of the
33 nitrocellulose strips in a horizontal line to form the
34 capture zone 32. The strips were then air dried for 1
35 hour at 37°C.

36 Free absorption sites were then blocked by
37 incubating the strips for 2 hours in a solution of 1%
38 gelatin (Norland Products Inc., New Brunswick Canada),

1 and 0.05% Tween 20 (Sigma) in PBS. The nitrocellulose
2 strips were then briefly washed in water, dried for one
3 hour in an incubator at 37°C and stored under
4 desiccation for at least four months. A square of 0.5x
5 2 cm of Whatman 3MM paper was attached to the top of
6 the strip to serve as an absorbent pad 27.

7 2. Mylered nitrocellulose lengths were prepared as
8 above but without the blocking step.

9

10 d) Transport and concentration of the DNA

11 The PCR reaction mixture was diluted ten fold in
12 either TGP running buffer (0.30% Tween 20 and 1%
13 gelatin in PBS), or PBS. 30 µl of each solution were
14 then transferred to wells similar to the wells of
15 apparatus 12 shown in Figs. 1 - 4 and the contact
16 portion 20 of each strip 22 was brought into contact
17 with the solutions.

18 The solution was allowed to migrate through the
19 nitrocellulose strips forming the bibulous carrier 24
20 at room temperature for 10 minutes. The strips 22 were
21 then covered completely by a solution of streptavidin
22 alkaline phosphatase conjugate (Enzymatix, Cambridge,
23 U.K.) diluted 1:2,500. After a 10 minute incubation at
24 room temperature the strips were washed briefly with
25 water and then covered by BCIP/NBT ChemiProbe™
26 solution (Organics Ltd.). After 5 minutes the strips
27 were briefly washed with water and inspected. The color
28 was then stabilized by a brief washing in ethanol and
29 then dried at room temperature. A strip 22 was
30 considered positive for HIV if a purple line appeared
31 in the capture zone.

32 Running the HIV product of PCR amplification on
33 nitrocellulose strips using PBS as a buffer wherein
34 the absorption sites of the nitrocellulose strips were
35 not blocked failed to produce a positive reaction. The
36 strips 22, however, in which the free absorption sites
37 of the nitrocellulose were blocked by gelatin solution
38 produced a visible signal when PBS was used as a

1 running buffer. In addition, the strips 22, wherein the
2 absorption sites were not blocked prior to their
3 contact with PCR reaction mixture solutions produced a
4 visible signal when the TGP running buffer was used.
5 The strongest signal was obtained when both a blocked
6 strip and the TGP running buffer were used.

7 These results indicate that amplified nucleic acid
8 sequences can migrate by capillary movement through
9 nitrocellulose strips wherein the absorption sites of
10 the nitrocellulose are blocked either prior to or
11 during the capillary transport of the nucleic acid
12 sequences. Moreover these results also indicate that
13 amplified DNA in a solution may be concentrated by
14 contacting blocked nitrocellulose strips at a contact
15 point with a solution containing amplified DNA and
16 capturing the amplified DNA at an appropriate capture
17 site on the nitrocellulose strip downstream of the
18 contact point.

19

20

21

22

EXAMPLE 2TRANSPORT AND CONCENTRATION OF GENOMIC AND PLASMID DNA
ON NITROCELLULOSE

25

26 Human Placenta DNA (Sigma), CasKi cells DNA and
27 Bluescript plasmid DNA were prepared and sulfonated as
28 described by Nur et al. (Ann. Biol. Clin., 1989, 47,
29 601 - 606) with each molecule of CasKi cell DNA or
30 Human Placental DNA having about 10^{15} base pairs. HIV
31 specific PCR products were amplified with one primer
32 being sulfonated another primer being biotinylated,
33 thus double labeling the PCR products as described in
34 Example 1. The nitrocellulose strips 22 having blocked
35 absorption sites were also prepared as described in
36 Example 1.

37 One μ l of a 20 μ g/ml solution of each of the three
38 types of DNA (either sulfonated or unsulfonated) was

1 added to 20 μ l of TGP running buffer. The DNA solution
2 was loaded into wells and the contact portion 20 of the
3 strips 22 brought into contact with this solution.
4 After 10 minutes the strips were removed from the DNA
5 solution and transferred to other wells where the
6 contact portion 20 of the strips 22 was brought into
7 contact with double label PCR product (diluted 1:20
8 from the HIV PCR reaction mixture solution of Example
9 1) and streptavidin alkaline phosphatase conjugate
10 (Enzymatix, Cambridge, U.K.) diluted 1:2,500 in TGP
11 running buffer. After 10 minutes of contact with the
12 double label DNA product the strips 22 were washed for
13 10 minutes by contacting the contact portion of strips
14 22 with a washing solution of TGP buffer. Finally, the
15 strips 22 were immersed in a ChemiProbeTM BCIP/NBT
16 solution (commercially available from Organics Ltd.)
17 for a 5 minute incubation period as described in
18 Example 1.

19 It was found that all three types of DNA,
20 Placental DNA, CasKi cell DNA and Bluescript plasmid
21 DNA, when sulfonated completely prevent the development
22 of a visible signal in the capture zone 32. In contrast
23 to these results, solutions containing the same DNA,
24 but where the DNA was not sulfonated failed to inhibit
25 the signal. These results indicate that both genomic
26 DNA and plasmid DNA can be transported by capillary
27 movement of a liquid through a nitrocellulose carrier
28 and that this DNA can be concentrated at an appropriate
29 capture site on the nitrocellulose strip.

30 The above results also suggest that the presence
31 of target DNA in a sample can be detected by the
32 reduction in signal produced by the double label PCR
33 product when target DNA is sulfonated and bound to
34 the capture zone 32 before capturing the double label
35 DNA as described above.

36

37

EXAMPLE 3

38

COMPARISON OF DETECTION SYSTEMS

1 Primers were selected in the E6 gene of the HPV
2 genome and were consensus primers for HPV 16, HPV 18
3 and HPV 33 described in Israel Patent Application No.
4 097226 the teachings of which are herein incorporated
5 by reference. These primers had the following
6 sequences:

7

8 Primer h15' AAGGGAGTAACCGAAATCGGT

9 Primer h25' ATAATGTCTATATTCACTAATT

10

11 The primer synthesis and labeling procedure was
12 described in Example 1. Primer h1 was sulfonated and
13 Primer h2 biotinylated according to these procedures.

14

15 Amplification and labeling of HPV DNA SEQUENCE

16

17 100 μ l of reaction mixture containing 100 pmole of
18 labeled or unlabeled primers, 1 μ g of DNA extracted
19 from cervical biopsies according to the instructions of
20 the HybriComb™ HPV kit (commercially available from
21 Organics Ltd.), 0.25 mM of deoxynucleotide triphosphate
22 (dNTP), 10 μ l 10X Taq buffer (commercially available
23 from Promega), and 2.5 units of Taq polymerase
24 (commercially available from Promega). The
25 thermocycling of the mixture was performed with a Grant
26 programmable water bath.

27 A first PCR step was performed using the unlabeled
28 primers. Each amplification cycle consisted of: DNA
29 denaturing for 1 minute at 94°C, annealing step 1
30 minute at 55 °C, and DNA extension step for 1 minute at
31 72°C. The amplification reaction was terminated by 5
32 minutes of extension at 72°C after 20 cycles. A second
33 PCR step using labeled primers was performed according
34 to the following procedure. One μ l of the first
35 reaction mixture was added to each of six replicates
36 containing 100 μ l of reaction mixture identical to
37 that of the first PCR reaction (except that labeled
38 rather than non-label primers were used). Each

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1 replicate was amplified for either 0, 10, 20, 25, or 30
2 cycles and then stored at 4°C.

3

4 Detection of the PCR product

5 1. Detection by ethidium bromide - EtdBr.

6 After amplification, 10 µl of the PCR mixture was
7 electrophoresed on 8% non-denaturing (TAE) Tris-acetic
8 acid buffer polyacrylamide gel and electrophoresed for
9 1 hour at 50 mA. Gels were submerged for 15 min. in 10
10 µg/l of ethidium bromide (EtdBr) and DNA was visualized
11 by UV light.

12

13 2. Detection by Southern blot.

14 After separation by electrophoresis the migrated
15 PCR fragments were electroblotted onto Hybond-N
16 membrane (commercially available from Amersham, Bucks,
17 U.K.) using TAE buffer as the transfer buffer in a
18 Trans Blot Cell (Commercially available from Bio-Rad,
19 Richmond, CA, USA) for 3 hours at 1.5 Amp. The membrane
20 was then air dried and baked for 2 hours at 80°C.

21 Visualization of the biotinylated label was
22 performed as follows: The membrane was blocked by PBS
23 supplemented with 1-light (Tropix, MA, USA) and 0.1%
24 Tween 20. The nylon membrane was incubated for 1 hour
25 in the same blocker supplemented with streptavidin
26 alkaline phosphatase conjugate diluted 1:2500 and then
27 washed by a solution containing 0.1% Tween 20 in PBS.
28 Finally, the membrane immersed in a ChemiProbeTM
29 BCIP/NBT chromogenic solution for 30 minutes and the
30 excess chromogen rinsed with water.

31

32 3. Detection by solid support capture (dip-stick)
33 assay.

34 Non-bibulous impact polystyrene (commercially
35 available from Organics Ltd.) was used as a solid
36 support for a dip-stick type capture assay.

37 Preparation of the dip-stick. One microliter of a
38 solution of 2 mg/ml purified mouse monoclonal anti-

1 modified DNA in PBS was applied to the lower portion of
2 the dip-stick and then dried for 1 hour at 37°C. The
3 unbound sites were blocked by dipping the dip-stick
4 into a solution of 1% gelatin and 0.05% Tween 20 for 1
5 hour. The dip-sticks were then washed for 2 - 5 seconds
6 in water and dried at 37°C for 1 hour.

7 The assay:

8 5 μ l of a reaction mixture solution from each of
9 the second PCR cycle groups was added to 45 μ l of TGP
10 running buffer containing streptavidin alkaline
11 phosphatase conjugate (1:200). The solutions were
12 placed in wells and the dip-stick was dipped into the
13 solutions. After 30 minutes incubation the dip-sticks
14 were washed in PBS and dipped in BCIP/NBT solution for
15 20 minutes. The reaction was terminated by washing the
16 dip-sticks in water.

17

18 4. Detection by Capillary DNA Concentration Assay
19 (CDCA).

20 3 μ l of each of reaction mixture solution from
21 each of the second PCR cycle groups was added to wells
22 containing 30 μ l of solution containing streptavidin
23 alkaline phosphatase conjugate diluted 1:2,500 in TGP
24 running buffer. Nitrocellulose strips were prepared as
25 in Example 1. The contact portion 20 of the strips 22
26 were brought into contact with the solution in the
27 wells for 10 minutes. The contact portion of the strips
28 22 were then brought into contact for 10 minutes with
29 wells containing 50 μ l of washing solution (TP buffer).
30 Finally, the strips 22 were completely immersed in a
31 ChemiProbeTM BCIP/NBT solution for 5 minutes to provide
32 a substrate for a chromogenic reaction.

33 The results of the above procedures are present in
34 Table 1 which indicates the detection limit in
35 relation to the number of PCR cycles for the assays
36 described above - EtdBr, Southern blot, solid support
37 capture assay and CDCA.

38

Table 1

Detection Limit of Several Systems

Number of PCR cycles

0 10 15 20 25 30

6 System

7

8 Etd/br - - ± + + +

9 South

10 blot

11

12

13

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5

1

33

80

80

27

72

As can be seen from Table 1 the sensitivity of the dip-stick test is similar to that of the EtdBr fluorescence test, both of which are less sensitive than the Southern Blot technique. The CDCA was seen to be at least as sensitive as the Southern blot technique.

29

10

EXAMPLE 4

32 EFFECT OF PRIMER ELIMINATION AFTER AMPLIFICATION ON THE
33 SENSITIVITY OF THE CDCA PROCEDURE

34 Specific HIV sequences were amplified from a
35 positive HIV sample in a 100 μ l reaction mixture for 20
36 PCR cycles using 100 pmole of unlabeled Primer 3 and
37 Primer 4 as described in Example 1. The second
38 amplification was performed under the same conditions

1 as the first amplification but with labeled primers and
2 for 2, 4, 6, 8, 10, and 20 cycles. The template for the
3 second PCR amplification was 1 μ l of the first PCR
4 mixture diluted in 100 μ l of reaction mixture
5 containing 100 pmoles of each labeled primer, 0.25 mM
6 of the four deoxynucleotide triphosphate, 10 μ l of 10X
7 Taq buffer (Promega) and 2.5 U of Taq polymerase
8 (Promega). For each PCR amplification cycle number
9 group, 4 aliquots of 100 μ l of PCR reaction mixture
10 were tested, one for each assay .

11

12 Assay-1.

13 The first assay was the CDCA system described in
14 Example 3. From each PCR amplification cycle number
15 group, 3 μ l of reaction mixture were added to wells
16 containing 30 μ l streptavidin alkaline phosphatase in
17 TGP running buffer and the CDCA was performed as
18 described in Example 3.

19

20 Assay-2

21 In the second assay the PCR reaction mixture was
22 treated with PEG to remove primers before running the
23 CDCA. Primers of each PCR amplification cycle number
24 group were excluded using a PEG solution as described
25 in Example 1. 3 μ l of the PEG treated PCR amplification
26 mixture was added to 30 μ l of TGP running buffer and
27 the assay then performed as in Example 3.

28

29 Assay-3

30 In the third assay primers in the PCR reaction
31 mixture were excluded by Sephadex G-100 prior to CDCA.
32 Primers of each of the PCR amplification cycle number
33 group were excluded by Sephadex G-100 as follows. 0.5
34 ml of Tris EDTA buffer (TE) in Sephadex G-100
35 (Pharmacia) was transferred to a well, excess TE was
36 absorbed by filter paper. 15 μ l of each PCR reaction
37 mixture solution was diluted 1:1 with TGP running
38 buffer and the mixture placed directly in the bottom of

1 the well.

2 The contact portion 20 of a strip 22, including a
3 strip of nitrocellulose wherein the absorption sites
4 are blocked was prepared as in Example 1, was brought
5 into contact with the upper side of the Sephadex G-100
6 for 25 minutes. The contact portion of the strip 22 was
7 then brought into contact for 10 minutes with
8 streptavidin alkaline phosphates conjugate diluted
9 1:2,500 in TGP running buffer in a well, then washed
10 and visualized according to the procedure of Example 1.

11

12 Assay-4

13 In the fourth assay primers were removed from the
14 PCR reaction mixture prior to the CDCA by hybridization
15 of the primers to complementary oligonucleotide
16 sequences bound to a compound. Primers of each PCR
17 amplification cycle number group were trapped by being
18 brought into contact with beads coated with
19 oligonucleotides having sequences complementary to the
20 sequences of the primers to be trapped.

21

22 a) Preparation of the trapping system. Streptavidin
23 was bound to styrene/vinyl carboxylic acid beads (5 μ m
24 in diameter commercially available from Bangs
25 Laboratories, Inc. Carmel, IN, USA) according to the
26 principles of Woodward, R.B. and Elofson, R.A. (1961).
27 J. Amer. Chem. Soc. 83, 1007-1010 under conditions
28 described in Israel Patent Application 098452, the
29 teachings of which are herein incorporated by
30 reference. The complementary oligonucleotide sequence,
31 5' TATTCCTAATTGAACTTCAA was synthesized and
32 biotinylated as described in Example 1.

33 The oligonucleotide was bound to the beads by the
34 following procedure. 100 μ l of 1% coated beads were
35 mixed 1:1 with a solution of 1mg/ml of biotinylated
36 oligonucleotide. The solution was incubated for 3 hours
37 at 30°C. The unbound oligonucleotide was washed in PBS
38 and kept in a solution of 1% gelatin in PBS.

1

2 b) The assay procedure 3 μ l of each PCR amplification
 3 cycle number group was added to wells containing 30 μ l
 4 of a solution containing 0.50% complementary
 5 oligonucleotide coated beads and streptavidin alkaline
 6 phosphatase conjugate (diluted 1:500) in TGP buffer and
 7 allowed to incubate for 10 minutes.

8 A contact portion 20 of strip 22, including a
 9 nitrocellulose strip wherein the absorbent sites were
 10 blocked and was prepared as in Example 1, is then
 11 brought into contact with the incubated solution for 10
 12 minutes. The strip 22 was then washed and the signal
 13 developed as in Example 3.

14 Table 2 shows the effect of elimination of primers
 15 after amplification on the sensitivity of the CDCA.

16

17 **Table 2**
 18 **Detection Limit of Assays 1-4**
 19 **Number of PCR cycles**

	0	2	4	6	8	10	20
--	---	---	---	---	---	----	----

21 **System**

22 -----

23 **Assay 1** + +

24

25 **Assay 2** + + + + + +

26

27 **Assay 3** + + + + + +

28

29 **Assay 4** + + + + + +

30

31 -----

32

33 + = detection of the HIV DNA sequences.

34 As can be seen from Table 2 untreated PCR solution
 35 fails to provide a visible signal in the CDCA assay
 36 even after 8 cycles of amplification. Only after some
 37 10 cycles does a positive response appear. Elimination
 38 of the primers after amplification by a separation

1 stage or during the test enables the detection of
2 target nucleic acid sequences after only 2 - 6 PCR
3 cycles. Elimination of primers by each technique has
4 been confirmed by gel electrophoresis and visualization
5 by EtdBr (data not shown).

6

7 EXAMPLE 5

8

9 DETECTION OF HPV SEQUENCES IN CLINICAL SAMPLES BY
10 HYBRIDIZATION IN SOLUTION

11

12 Preparation of the probe. A single stranded HPV sequence
13 was prepared by asymmetric PCR amplification using the
14 HPV primer h1 described in Example 3. The following
15 conditions for amplification were employed. 10 ng of
16 non-labeled HPV PCR product prepared as described in
17 example 3 was used as a template and only one primer h1
18 was used for amplification. 50 PCR cycles were
19 performed as described in Example 3.

20 The single stranded product was then sulfonated
21 for one hour at 30°C and was then desalting by using
22 Sephadex G-50 as described in the instructions for the
23 use of the ChemiProbe™ kit (Organics, Ltd.)

24

25 Amplification of the HPV Sequence

26 The HPV sequences were amplified from a clinical
27 sample by two methods: A) using biotinylated h2 primers
28 and non labeled h1 primers and B) using biotinylated
29 h2 primers and sulfonated h1 primers. For both methods
30 PCR was performed as described in Example 3 for 35
31 cycles.

32

33 Hybridization

34 5 µl of the PCR reaction mixture solution of
35 method A (after 35 cycles) was added to 95 µl of a
36 hybridization solution containing 0.66M NaCl, 65mM
37 sodium citrate, 0.3 mM EDTA, 0.1M phosphate buffer pH
38 6.6, 0.02% Ficoll™, 0.2% Polyvinylpyrrolidone, 0.5%

1 Polyethylglycol, 0.12% bovine serum albumin, and 100 ng
2 of a sulfonated probe described above. The solution was
3 then heated for 5 minutes at 95°C and cooled
4 immediately. Hybridization was performed for 45 minutes
5 at 65°C.

6

7 Capture by CDCA

8 3 μ l of the hybridization mixture after completion
9 of the hybridization or 0.3 μ l of PCR reaction mixture
10 solution from method B were added to wells containing
11 30 μ l of streptavidin alkaline phosphatase in TGP
12 running buffer. A contact portion 20 of strip 22,
13 including a nitrocellulose strip which was prepared as
14 in Example 1, was then brought into contact for 10
15 minutes with the solution in the well, the hybrid was
16 captured and visualized as in Example 3.

17

18 Results

19 Twelve samples were evaluated. The same 5 samples were
20 found positive and the same 7 samples found negative
21 for both methods tested.

22

23 EXAMPLE 6

24 DETECTION OF HPV IN THE CDCA SYSTEM USING COLORED LATEX
25 BEADS AS THE COLOR GENERATING REAGENT

26 Streptavidin (Sigma) was covalently bound to 0.2 μ m
27 styrene/vinyl carboxylic acid colored beads (Bangs
28 Laboratories Inc., Carmel, IN, USA). The binding was
29 accomplished by the methods of Woodward et al. as
30 described in Example 4.

31 PCR product from a clinical sample suspected to
32 contain HPV sequences were amplified by a second PCR
33 amplification step using h-1 sulfonated and h-2
34 biotinylated primers as described in Example 3. Primers
35 were excluded from the PCR reaction mixture solution
36 using PEG solution as described in Example 1. 3 μ l of
37 this solution was added to a well containing 0.05% of
38 streptavidin bound beads in 1.0% gelatin, 0.3% Tween 20

1 and 0.25 M NaCl. The contact portion 20 of a strip 22
2 prepared as described in Example 3 was placed in the
3 well, in contact with the solution in the well. After a
4 few minutes a blue colored signal was visible in the
5 capture zone 32 of the strip 22.

6

EXAMPLE 7

8 DETECTION OF HPV SEQUENCES IN A CAPILLARY DNA
9 CONCENTRATION ASSAY USING DNA AS A CAPTURE REAGENT

10

11 a) Selection of primers

12 Primers were selected in the E6 gene of HPV/16 and had
13 the following sequences:

14

Primer 1

15

GTAACCGAAATCGGT

16

Primer 2

38

5' CTTCTTGGGACCTCTCTGC

10

30

31 b) Oligonucleotide probe capture reagent

22 The oligonucleotide probe which serves as a
23 capture reagent was selected to be complementary to the
24 sequence of a biotinylated strand produced by the
25 elongation of primer 2 in a PCR reaction. The following
26 sequence was chosen:

27 CAACAAACAACAAGTTCAGGACCCACAGGAGCGACCC

28

29 c) Preparation of the Nitrocellulose backed strips

30 Mylered nitrocellulose, pore size 5 microns,
31 (Micron Separation Inc., Westboro, MA, USA) was cut
32 into 0.5 x 3.0 cm strips. One microliter of a solution
33 composed of 5 ng oligonucleotide probe capture reagent
34 in 10X SSC (SSC consisting of 0.15M NaCl and 0.015M
35 sodium citrate, pH 7.0) was applied to middle of each
36 nitrocellulose strip forming a spot. The strips were
37 then dried for 15 minutes at 37°C and the
38 oligonucleotide probes were then fixed to the

1 nitrocellulose strips by exposure of the strips to UV
2 radiation for 5 minutes.

3

4 d) Amplification of the HPV sequence

5 PCR amplification was performed in a reaction
6 mixture of 100 μ l aliquots containing either 1,000,
7 100, 10, 1 or 0 pg of CasKi cell DNA in the presence of
8 1 μ g normal human placenta DNA. Each PCR reaction mix
9 additionally contained 100 pmole of each of the primers
10 (P1 and P2), 0.25mM of the four deoxynucleotide
11 triphosphates, 10 μ l 10X Taq buffer and 2.5 U of Taq
12 DNA polymerase.

13 A first DNA denaturing step of 5 minutes at 94°C
14 was followed by 30 cycles of 1 minute denaturing at
15 94°C, 1.5 minute annealing at 47°C. and 1.5 minute
16 elongation at 72°C. The amplification was ended with a
17 seven minute elongation at 72°C.

18 e) Transport and concentration of DNA

19 The concentration and capturing of target nucleic
20 acid sequences was achieved by the following
21 chromatography hybridization procedure:

22 50 μ l of each PCR product obtained in step d above
23 was diluted 1:10 in 450 μ l of hybridization solution
24 composed of 0.6M NaCl, 20mM phosphate buffer, pH 7.5,
25 0.02% Ficoll 400 (Sigma, St. Louis, MO, USA), 0.02%
26 gelatin and 1% PVP. The samples were boiled for 10
27 minutes and chilled immediately on ice. 200 μ l of each
28 solution was then transferred to the wells 14 of the
29 apparatus 12 shown in Figs. 1-4 and the contact portion
30 20 of each strip 22 was brought into contact with the
31 solution in the wells 14.

32 The apparatus 12 was placed in a humid incubator
33 (90% relative humidity) at 37°C for 25 minutes and the
34 solution was allowed to migrate through the
35 nitrocellulose strips forming the bibulous carrier 24.
36 The strips 22 were then transferred to wells 16
37 containing 100 μ l of streptavidin alkaline phosphatase
38 conjugate diluted 1:2,500 in PBS and 0.3% Tween 20 for

1 20 minutes. The strips 22 were then transferred to
2 wells containing a solution including 150 μ l PBS and
3 0.3% Tween 20. The contact portion 20 of the strip 22
4 was brought into contact with the solution for 15
5 minutes at 37°C. Finally the strips 22 were completely
6 immersed in a ChemiProbeTM BCIP/NBT solution for 20
7 minutes at 37°C to provide a substrate for a
8 chromogenic reaction. A blue colored signal in the
9 capture zone 32 of strip 22 indicating the presence of
10 HPV DNA.

11 It was found that HPV sequences existing in as low
12 as 1 pg CasKi DNA can be detected by this
13 chromatography hybridization procedure.

14 It will be appreciated by persons skilled in the
15 art that the present invention is not limited to what
16 has been particularly shown and described herein above.
17 Rather the scope of the present invention is defined
18 only by the claims which follow:

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CLAIMS

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4 1. Apparatus for transport of molecules including
5 nucleic acid sequences in a bibulous carrier comprising
6 a dry bibulous carrier defining a capillary transport
7 path which supports the transport of the molecules when
8 contacted with a solution containing the molecules.

9

10 2. Apparatus according to claim 1 for concentration
11 of target molecules in a liquid sample comprising:

12 the dry bibulous carrier wherein the target
13 molecules include target nucleic acid sequences and are
14 transported within the bibulous carrier by capillary
15 action when a portion of the dry bibulous carrier
16 contacts the liquid sample containing the target
17 molecules; and

18 at least one capture reagent immobilized in
19 at least one capture zone on the dry bibulous carrier
20 downstream of a contact portion of the bibulous carrier
21 wherein the at least one capture reagent is capable of
22 capturing the target molecules.

23

24 3. Apparatus for separation of target molecules,
25 including target nucleic acid sequences, from non-
26 target nucleotides and oligonucleotides in a liquid
27 sample containing the target molecules and the non-
28 target nucleotides and oligonucleotides comprising:

29 a vessel containing a compound that binds the
30 non-target oligonucleotides; and

31 means for transporting the target molecules
32 from the vessel by capillary action.

33

34 4. Apparatus according to claim 2 wherein the dry
35 bibulous carrier is a nitrocellulose membrane wherein
36 the absorption sites have been blocked to facilitate
37 capillary transport of the target molecules.

38

1 5. Apparatus according to claim 4 wherein the dry
2 bibulous carrier is supported by a rigid frame.

3

4 6. Apparatus according to claim 2 wherein an
5 absorbent pad is fixed to the dry bibulous carrier
6 downstream from the at least one capture zone to
7 facilitate capillary transport of a liquid through the
8 dry bibulous carrier.

9

10 7. Apparatus according to claim 4 wherein the
11 absorption sites of the nitrocellulose membrane are
12 blocked by compounds selected from a group comprising
13 macromolecules, detergents and combinations thereof.

14

15 8. Apparatus according to claim 7 wherein the
16 macromolecules include proteins.

17

18 9. Apparatus according to claim 2 wherein the at
19 least one capture reagent comprises an antibody to a
20 modified portion of the target nucleic acid sequence.

21

22 10. Apparatus according to claim 2 wherein the at
23 least one capture reagent comprises at least one
24 nucleic acid capture reagent including nucleic acid
25 probe sequences complementary to at least part of the
26 target nucleic acid sequences.

27

28 11. Apparatus according to claim 10 wherein the
29 nucleic acid probe sequences include DNA sequences.

30

31 12. Apparatus according to claim 10 wherein the
32 nucleic acid probe sequences include RNA sequences.

33

34 13. Apparatus according to claim 1 wherein the target
35 molecules include target nucleic acid sequences
36 comprising more than 30 base pairs.

37

38 14. Apparatus according to claim 2 wherein the target

1 molecules including nucleic acid sequences comprise a
2 nucleic acid product of an enzymatic amplification
3 reaction and incorporate at least one pair of
4 oligonucleotide primers.

5

6 15. Apparatus according to claim 14 wherein the at
7 least one pair of primers comprise primers for a
8 polymerase chain reaction (PCR).

9

10 16. Apparatus according to claim 14 wherein the at
11 least one pair of primers comprise primers for a
12 ligase chain reaction (LCR).

13

14 17. Apparatus according to claim 14 wherein at least a
15 second primer of the at least one pair of primers
16 includes an oligonucleotide bearing a ligand which
17 binds to a at least one capture reagent whereby the
18 target molecules which include the at least one primer
19 bearing the ligand may be bound to the at least one
20 capture reagent.

21

22 18. Apparatus according to claim 17 wherein the
23 ligand comprises an antigenic epitope.

24

25 19. Apparatus according to claim 18 wherein the ligand
26 comprises at least one sulfonated cytosine.

27

28 20. Apparatus according to claim 3 wherein the non-
29 target oligonucleotides comprise oligonucleotide
30 primers not incorporated in the target nucleic acid
31 sequences.

32

33 21. Apparatus according to claim 3 wherein the
34 compound comprises gel filtration particles too large
35 to be transported by the means for transporting.

36

37 22. Apparatus according to claim 3 wherein the
38 compound comprises a matrix unable to be transported by

1 the means for transporting and wherein the compound
2 hybridizes to the non-target oligonucleotide.

3 23. A method for transport of molecules including
4 nucleic acid sequences in a bibulous carrier comprising
5 the steps of:

6 providing a dry bibulous carrier defining a
7 capillary transport path which supports the transport
8 of molecules including nucleic acid sequences; and
9 contacting the dry bibulous carrier with a
10 solution containing molecules including nucleic acid
11 sequences.

12

13 24. A method for concentration of molecules, including
14 nucleic acid sequences, in a liquid sample comprising
15 the steps of:

16 providing a dry bibulous carrier wherein the
17 molecules are target molecules including target nucleic
18 acid sequences and wherein the molecules are
19 transported within the bibulous carrier by capillary
20 action when a portion of the dry bibulous carrier
21 contacts the liquid sample containing the molecules;

22 contacting a portion of the dry bibulous
23 carrier with the liquid sample containing the target
24 molecules wherein the dry bibulous carrier, when wet,
25 defines a liquid transport path which supports the
26 transport of molecules including nucleic acid
27 sequences;

28 transporting the target molecules along the
29 liquid transport path; and

30 capturing the target molecules with at least
31 one capture reagent immobilized in at least one
32 capture zone on the dry bibulous carrier downstream of
33 the portion of bibulous carrier contacting the liquid
34 sample.

35

36 25. A method for separation of target molecules,
37 including target nucleic acid sequences, from non-
38 target nucleotides and oligonucleotides, in a liquid

1 sample containing the target molecules and the non-
2 target nucleotides and oligonucleotides comprising the
3 steps of:

4 providing a vessel containing a compound that
5 binds the non-target oligonucleotides;
6 adding the liquid sample which includes the
7 target molecules and the non-target nucleotides and
8 oligonucleotides; and
9 transporting the target molecules by
10 capillary action.

11

12 26. Apparatus for separation of target molecules,
13 including target nucleic acid sequences, from non-
14 target nucleotides and oligonucleotides in a liquid
15 sample containing the target molecules and the non-
16 target nucleotides and oligonucleotides, concentration
17 of the target molecules, and detection of the
18 concentrated target molecules comprising:

19 a vessel apparatus defining a plurality of
20 wells including a first portion of the plurality of
21 wells containing a compound that binds the non-target
22 oligonucleotides and wherein the liquid sample may be
23 added to the first portion of the plurality of wells;

24 a dry bibulous carrier defining a liquid
25 transport path from the vessel that when wet supports
26 the transport of the target molecules wherein the
27 target molecules are transported within the bibulous
28 carrier by capillary action when a contact portion of
29 the dry bibulous carrier contacts the liquid sample
30 containing the target molecules;

31 at least one capture reagent capable of
32 capturing the target molecules wherein the at least
33 one capture reagent is immobilized in at least one
34 capture zone on the dry bibulous carrier downstream of
35 the contact portion of the bibulous carrier; and

36 means for detecting the captured target
37 molecules.

38

1 27. A method for concentration and detection of
2 target nucleic acid sequences, in a liquid sample
3 comprising the steps of:

4 providing a dry bibulous carrier wherein the
5 target nucleic acid sequences are transported within
6 the bibulous carrier by capillary action when a portion
7 of the dry bibulous carrier contacts the liquid sample
8 containing the target nucleic acid sequences;

9 contacting a portion of the dry bibulous
10 carrier with the liquid sample containing the target
11 nucleic acid sequences wherein the dry bibulous
12 carrier, when wet, defines a liquid transport path
13 which supports the transport of the target nucleic acid
14 sequences;

15 transporting the target nucleic acid
16 sequences along the liquid transport path; and

17 capturing the target nucleic acid sequences
18 by hybridization with at least one nucleic acid capture
19 reagent immobilized in at least one capture zone on the
20 dry bibulous carrier downstream of the portion of
21 bibulous carrier contacting the liquid sample.

22

23 28. Apparatus for concentration and detection of
24 target nucleic acid sequences comprising:

25 a vessel apparatus defining a plurality of wells;

26 a dry bibulous carrier defining a liquid
27 transport path from the vessel that when wet supports
28 the transport of the target nucleic acid sequences
29 wherein the target nucleic acid sequences are
30 transported within the bibulous carrier by capillary
31 action when a contact portion of the dry bibulous
32 carrier contacts the liquid sample containing the
33 target nucleic acid sequences;

34 at least one nucleic acid capture reagent
35 including nucleic acid probe sequences for capturing
36 the target nucleic acid sequences by hybridization and
37 wherein the at least one nucleic acid capture reagent
38 is immobilized in a capture zone on the dry bibulous

1 carrier downstream of the contact portion of the
2 bibulous carrier; and
3 means for detecting the captured the target
4 nucleic acid sequences.

5

6 29. Apparatus according to claim 26 wherein the means
7 for detecting comprises:

8 a bibulous carrier upon which target
9 molecules, including nucleic acid sequences, bearing a
10 ligand which binds to a signal producing reagent are
11 immobilized; and

12 means for contacting the target molecules,
13 including the nucleic acid sequences, bearing the
14 ligand with the signal producing reagent to produce a
15 sensible signal indicating the detection of the target
16 molecules including the nucleic acid sequences.

17

18 30. Apparatus according to claim 29 wherein the target
19 nucleic acid sequences are the product of an enzymatic
20 amplification reaction and incorporate at least one
21 pair of oligonucleotide primers.

22

23 31. Apparatus according to claim 26 wherein the non-
24 target oligonucleotides comprise oligonucleotide
25 primers not incorporated in the target nucleic acid
26 sequences.

27

28 32. Apparatus according to claim 30 wherein the at
29 least one pair of primers comprise primers for a
30 polymerase chain reaction
31 (PCR).

32

33 33. Apparatus according to claim 30 wherein the one
34 pair of primers comprise primers for a ligase chain
35 reaction (LCR).

36

37 34. Apparatus according to claim 30 where a second
38 primer of the at least one pair of oligonucleotide

1 primers includes a ligand which binds to the at least
2 one capture reagent whereby the target molecules that
3 include the ligand may be bound to the at least one
4 capture reagent.

5

.6 35. Apparatus according to claim 34 wherein the ligand
7 comprises an antigenic epitope.

8

9 36. Apparatus according to claim 35 wherein the ligand
10 comprises at least one sulfonated cytosine.

11

12 37. Apparatus according to claim 30 where a first
13 primer of the at least one pair of primers includes a
14 ligand which binds to a signal producing reagent
15 whereby the target molecules that include the ligand
16 may be detected by the presence of a signal produced by
17 the signal producing reagent.

18

19 38. Apparatus according to claim 37 where a first
20 primer of the at least one pair of primers includes a
21 ligand which binds to a signal producing reagent
22 whereby the target molecules that include the ligand
23 may be detected by the presence of a signal produced by
24 the signal producing reagent after contacting a signal
25 developing reagent.

26

27 39. Apparatus according to claim 37 wherein the ligand
28 comprises biotinylated nucleotides.

29

30 40. Apparatus according to claim 37 wherein the signal
31 producing reagent comprises streptavidin linked to
32 colored latex beads.

33

34 41. Apparatus according to claim 38 wherein the signal
35 produced by the signal producing reagent after
36 contacting the signal developing reagent includes a
37 streptavidin-alkaline phosphatase conjugate.

38

1 42. Apparatus according to claim 26 wherein the first
2 portion of wells also contains the signal producing
3 reagent.

4

5 43. Apparatus according to claim 26 wherein the
6 plurality of wells additionally includes a second
7 portion of the wells containing a washing solution.
8

9 44. Apparatus according to claim 26 wherein the
10 plurality of wells also includes a third portion of the
11 wells containing a signal developing reagent solution.
12

13 45. Apparatus according to claim 28 wherein the
14 plurality of wells comprise a first portion of wells
15 containing a sample to be tested for the target nucleic
16 acid sequences.
17

18 46. Apparatus according to claim 28 wherein the
19 plurality of wells additionally comprises a second
20 portion of the wells containing the signal producing
21 reagent.
22

23 47. Apparatus according to claim 28 wherein the
24 plurality of wells additionally comprises a third
25 portion of wells containing a washing solution.
26

27 48. Apparatus according to claim 28 wherein the
28 plurality of wells additionally comprises a fourth
29 portion of wells containing a signal developing
30 reagent.
31

32 49. Apparatus according to claim 26 wherein the dry
33 bibulous carrier comprises at least one strip.
34

35 50. Apparatus according to claim 49 wherein each of
36 the first portion of wells are adapted to receive the
37 contact portion of each strip to permit transport of
38 the target molecules to the at least one capture zone

1 where they are captured.

2

3 51. Apparatus according to claim 43 wherein each of
4 the second portion of wells is adapted to receive the
5 contact portion of each strip for washing the strip to
6 remove no specifically captured compounds after
7 immobilization of the target molecules in the at least
8 one capture zone.

9

10 52. Apparatus according to claim 44 wherein each of
11 the third portion of wells is adapted to receive an
12 entire strip.

13

14 53. Apparatus according to claim 52 wherein the means
15 for contacting comprises:

16 at least one of the third portion of wells
17 containing a signal producing reagent solution; and
18 at least one strip after immobilization of
19 the target molecules in the at least one capture zone
20 wherein the entire strip is in contact with a signal
21 developing reagent solution permitting contact of the
22 signal developing reagent with the at least one capture
23 zone.

24

25 54. Apparatus according to claim 28 wherein each of
26 the first portion of wells is adapted to receive the
27 contact portion of each strip to permit transport of
28 the target nucleic acid sequences to the at least one
29 capture zone where they are captured.

30

31 55. Apparatus according to claim 46 wherein each of the
32 second portion of wells is adapted to receive the
33 contact portion of each strip to permit transport of
34 the signal producing reagent to the at least one
35 capture zone where the signal producing reagent is
36 bound to the ligand borne on the target nucleic acid
37 sequences.

38

1 56. Apparatus according to claim 47 wherein each of
2 the third portion of wells is adapted to receive the
3 contact portion of each strip for washing the strip to
4 remove non-specifically captured compounds after
5 immobilization of the target nucleic acid sequences in
6 the at least one capture zone.

7

8 57. Apparatus according to claim 48 wherein the means
9 for contacting comprises:

10 at least one of the fourth portion of wells
11 containing a signal developing reagent; and

12 at least one strip after immobilization of
13 the target nucleic acid sequences in the at least one
14 capture zone wherein the entire strip is in contact
15 with the signal developing reagent solution permitting
16 contact of the signal developing reagent with the at
17 least one capture zone.

18

19 58. Apparatus according to claim 57 wherein each of
20 the fourth portion of wells is adapted to receive an
21 entire strip.

22

23 59. A method for the detection of a specific nucleic
24 acid sequence comprising the steps of:

25 amplifying by an enzymatic reaction at least
26 a portion of an original nucleic acid sequence to
27 produce target molecules including nucleic acid
28 sequences which are specific to the at least a portion
29 of the original nucleic acid sequence;

30 separating the target molecules from non-
31 target nucleotides and oligonucleotides including the
32 steps of:

33 providing a vessel containing a substrate
34 that binds the non-target nucleotides and
35 oligonucleotides;

36 adding a liquid sample which includes the
37 target molecules and the non-target nucleotides and
38 oligonucleotide;

SUBSTITUTE SHEET

1 and transporting the target molecules by
2 capillary action;
3 concentrating the target molecules including
4 the steps of:
5 providing a dry bibulous carrier wherein the
6 target molecules are transported within the bibulous
7 carrier by capillary action when a portion of the dry
8 bibulous carrier contacts the liquid sample containing
9 the target molecules;
10 contacting a portion of the dry bibulous
11 carrier with the liquid sample containing the target
12 nucleic acid sequences wherein the dry bibulous
13 carrier, when wet, defines a liquid transport path
14 which supports the transport of the target molecules;
15 transporting the target molecules along the
16 liquid transport path; and
17 capturing the target molecules with at
18 least one capture reagent immobilized in a capture
19 zone on the dry bibulous carrier downstream of the
20 portion of bibulous carrier contacting the liquid
21 sample; and
22 detecting the target molecules by contacting
23 target molecules having a ligand which binds to a
24 signal producing reagent and are immobilized on a
25 bibulous carrier with a signal developing reagent to
26 produce a sensible signal.

27
28 60. A method for the detection of a specific nucleic
29 acid sequence comprising the steps of:

30 amplifying by an enzymatic reaction at least
31 a portion of an original nucleic acid sequence to
32 produce target nucleic acid sequences which are
33 specific to the at least a portion of the original
34 nucleic acid sequence;

35 providing a liquid sample which includes the
36 target nucleic acid sequences;

37 transporting the target nucleic acid
38 sequences by capillary action;

1 concentrating the target nucleic acid
2 sequences including the steps of:

3 providing a dry bibulous carrier wherein the
4 target nucleic acid sequences are transported within
5 the bibulous carrier by capillary action when a portion
6 of the dry bibulous carrier contacts the liquid sample
7 containing the target nucleic acid sequences;

8 contacting a portion of the dry bibulous
9 carrier with the liquid sample containing the target
10 nucleic acid sequences wherein the dry bibulous
11 carrier, when wet, defines a liquid transport path
12 which supports the transport of the target nucleic acid
13 sequences; and

14 transporting the target nucleic acid
15 sequences along the liquid transport path;

16 capturing the target nucleic acid sequences
17 with at least one nucleic acid capture reagent
18 immobilized in at least one capture zone on the dry
19 bibulous carrier downstream of the portion of bibulous
20 carrier contacting the liquid sample; and

21 detecting the target nucleic acid sequences
22 by contacting target nucleic acid sequences having a
23 ligand which binds to a signal producing reagent and
24 are immobilized on a bibulous carrier with a signal
25 developing reagent to produce a sensible signal.

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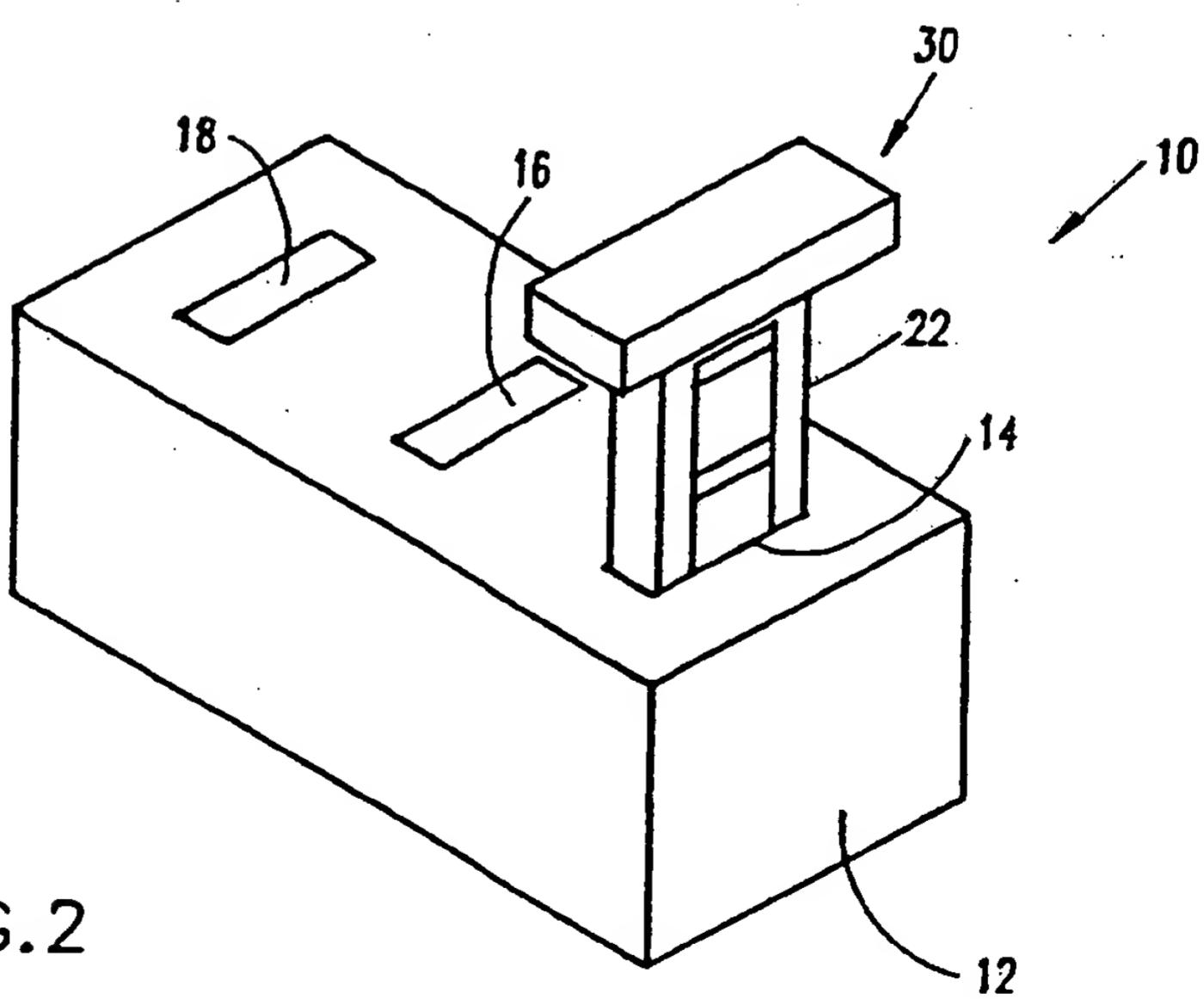
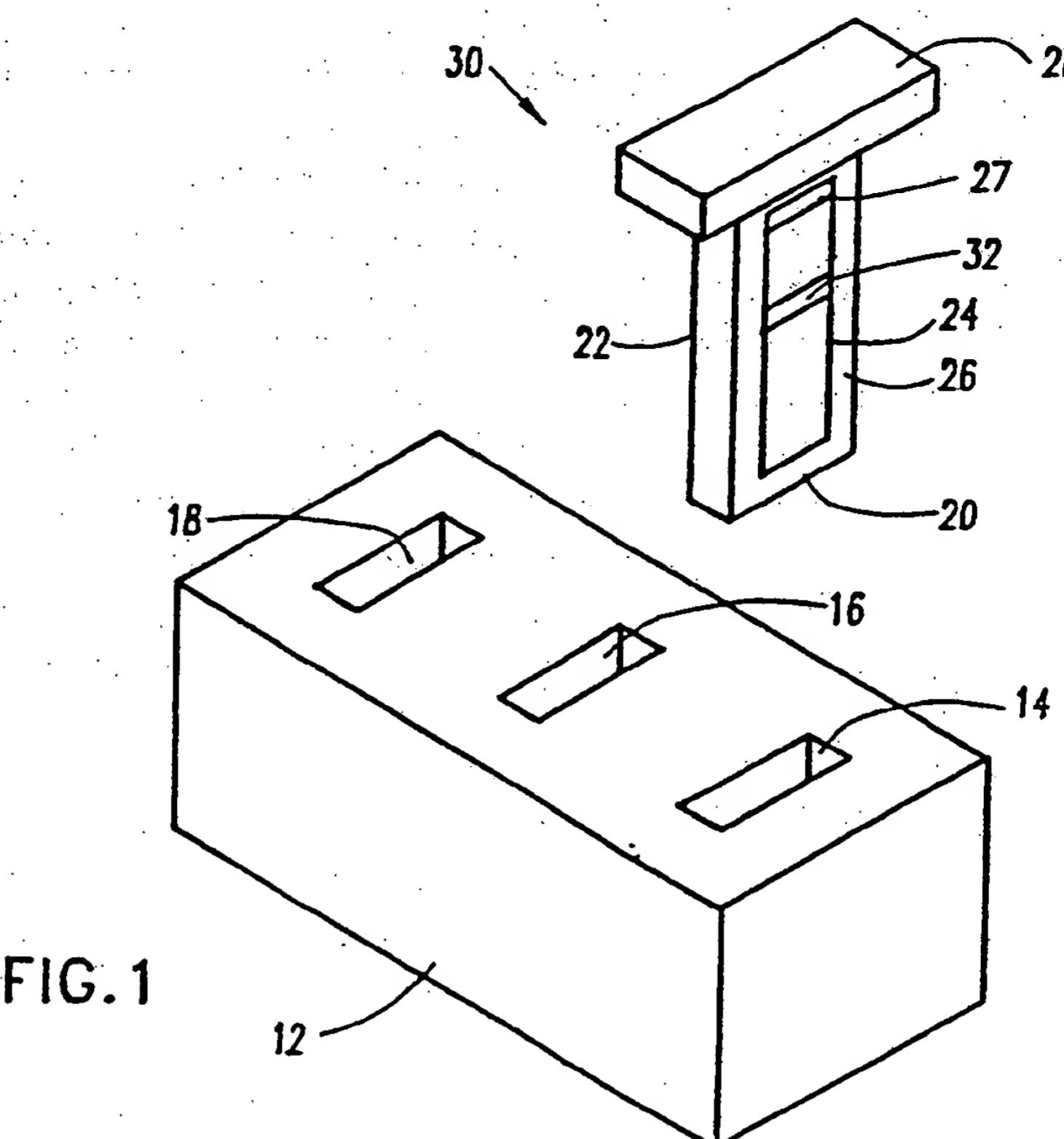
35

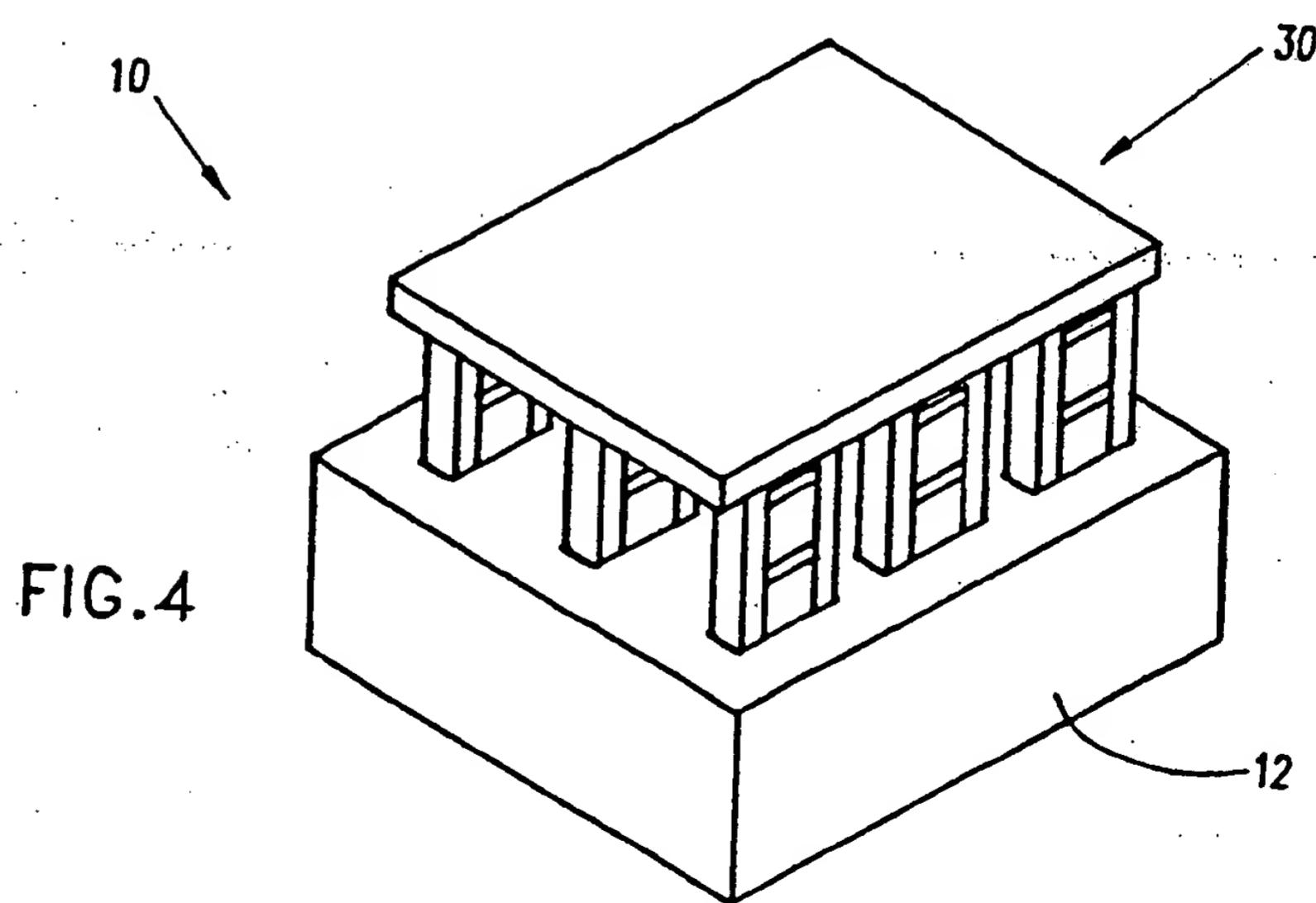
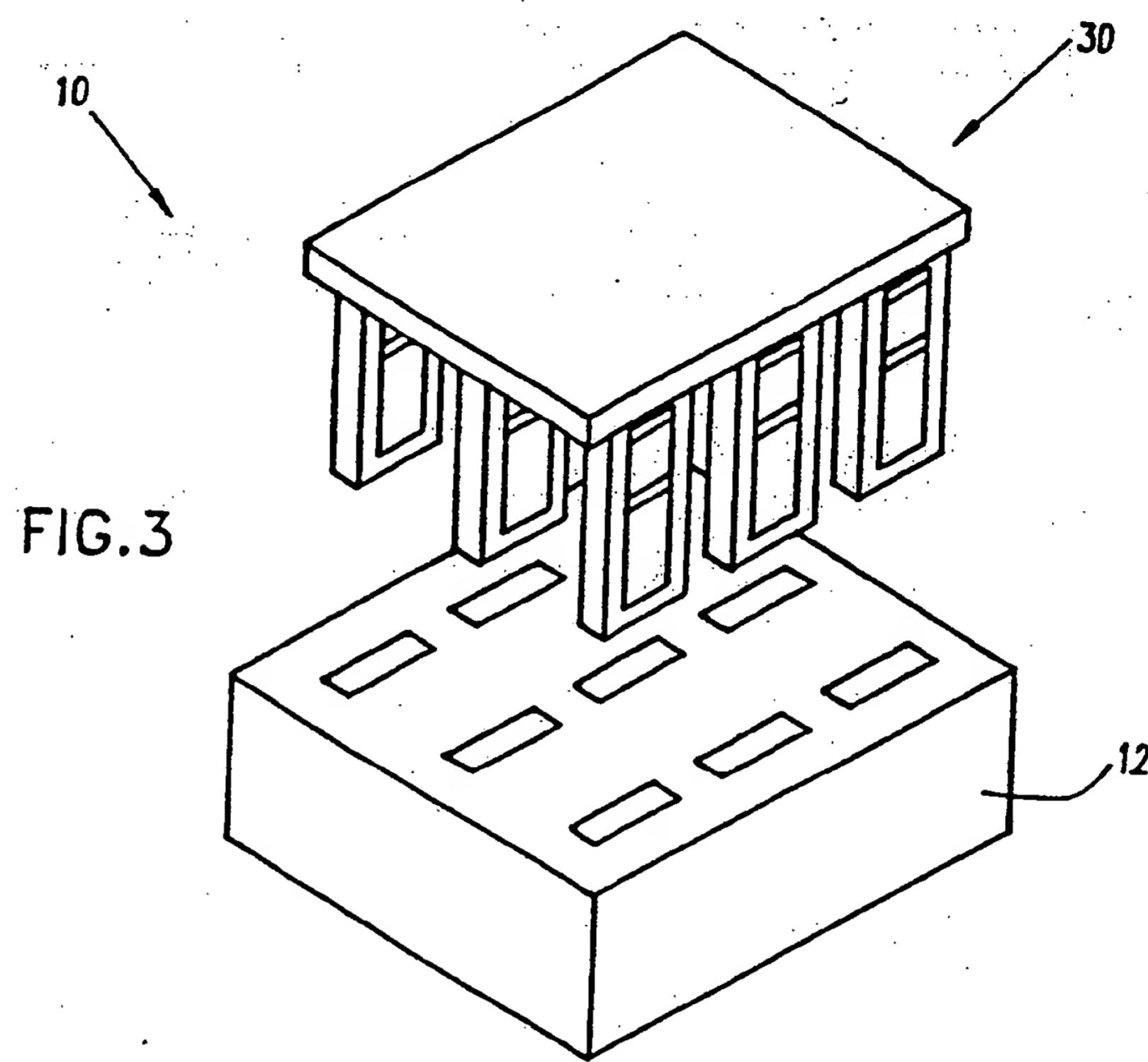
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/NL 92/00176

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.C1. 5 C12Q1/68; G01N33/558; G01N33/543;

// C12Q1/70

II. FIELDS SEARCHED

Minimum Documentation Searched⁷

Classification System	Classification Symbols
Int.C1. 5	C12Q ; G01N

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched⁸III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	EP,A,0 262 328 (ABBOTT LABORATORIES) 6 April 1988 see page 9, line 1 - page 15, line 52 see page 28, line 35 - line 47 see page 32, line 10 - page 33, line 52 see page 41, line 20 - page 42, line 50; claims --- EP,A,0 306 336 (SYNTEX (USA) INC.) 8 March 1989 see page 5, line 2 - line 19 see page 7, line 56 - page 8, line 18 see page 11, line 50 - page 12, line 39 see page 19, line 60 - page 21, line 61; claims ---	1-5, 23-25, 27, 49, 50 1 -/-
X		

¹⁰ Special categories of cited documents : 10¹¹ "A" document defining the general state of the art which is not considered to be of particular relevance¹² "E" earlier document but published on or after the international filing date¹³ "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)¹⁴ "O" document referring to an oral disclosure, use, exhibition or other means¹⁵ "P" document published prior to the international filing date but later than the priority date claimed¹⁶ "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention¹⁷ "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step¹⁸ "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art¹⁹ "A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

15 JANUARY 1993

Date of Mailing of this International Search Report

28. 01. 93

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

LUZZATTO E.R.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	<p>WO,A,8 910 979 (E.I. DU PONT DE NEMOURS AND COMPANY) 16 November 1989 see page 7, line 15 - page 16, line 16; claims ---</p>	2,14,17
A	<p>EP,A,0 318 255 (EASTMAN KODAK COMPANY) 31 May 1989 see the whole document ---</p>	29-32,60
A	<p>WO,A,9 106 659 (PBS-ORGENICS) 16 May 1991 see the whole document ---</p>	18,19, 26,36
A	<p>EP,A,0 362 809 (BOEHRINGER BIOCHEMIA ROBIN S.P.A.) 11 April 1990 see the whole document ---</p>	1-4
A	<p>GB,A,2 191 577 (LANCE ALLEN LIOTTA) 16 December 1987 see the whole document -----</p>	3,21,22

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

NL 9200176
SA 65378

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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15/01/93

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